

by 10 minutes at 72° C.; for NL016: 10 minutes at 95° C., followed by 40 cycles of 30 seconds at 95° C., 1 minute at 54° C. and 1 minute 40 seconds at 72° C., followed by 10 minutes at 72° C.; for NL018: 10 minutes at 95° C., followed by 40 cycles of 30 seconds at 95° C., 1 minute at 54° C. and 1 minute 35 seconds at 72° C., followed by 10 minutes at 72° C.; for NL019: 10 minutes at 95° C., followed by 40 cycles of 30 seconds at 95° C., 1 minute at 55° C. and 1 minute at 72° C., followed by 10 minutes at 72° C.; for NL021: 10 minutes at 95° C., followed by 40 cycles of 30 seconds at 95° C., 1 minute at 54° C. and 1 minute 45 seconds at 72° C., followed by 10 minutes at 72° C.; and for NL027: 10 minutes at 95° C., followed by 40 cycles of 30 seconds at 95° C., 1 minute at 54° C. and 1 minute 45 seconds at 72° C., followed by 10 minutes at 72° C. The resulting PCR fragments were analyzed on agarose gel, purified (QIAquick Gel Extraction kit, Cat. Nr. 28706, Qiagen), cloned into the pCR8/GW/topo vector (Cat. Nr. K2500 20, Invitrogen), and sequenced. The sequences of the resulting PCR products are represented by the respective SEQ ID NO:s as given in Table 2-NL and are referred to as the partial sequences. The corresponding partial amino acid sequences are represented by the respective SEQ ID NO:s as given in Table 3-NL.

B. Cloning of a Partial Sequence of the *Nilaparvata lugens* NL023 Gene Via EST Sequence

[0388] From high quality total RNA of *Nilaparvata lugens* (source: Dr. J. A. Gatehouse, Dept. Biological Sciences, Durham University, UK) cDNA was generated using a commercially available kit (SuperScript™ III Reverse Transcriptase, Cat N°. 18080044, Invitrogen, Rockville, Md., USA) following the manufacturer's protocol.

[0389] A partial cDNA sequence, NL023, was amplified from *Nilaparvata lugens* cDNA which corresponded to a *Nilaparvata lugens* EST sequence in the public database Genbank with accession number CAH65679.2. To isolate cDNA sequences comprising a portion of the NL023 gene, a series of PCR reactions with EST based specific primers were performed using PerfectShot™ ExTaq (Cat N°. RRO05A, Takara Bio Inc.) following the manufacturer's protocol.

[0390] For NL023, the specific primers oGBKWO02 and oGBKWO03 (represented herein as SEQ ID NO: 1157 and SEQ ID NO: 1158, respectively) were used in two independent PCR reactions with the following conditions: 3 minutes at 95° C., followed by 30 cycles of 30 seconds at 95° C., 30 seconds at 56° C. and 2 minutes at 72° C., followed by 10 minutes at 72° C. The resulting PCR products were analyzed on agarose gel, purified (QIAquick® Gel Extraction Kit; Cat. N°. 28706, Qiagen), cloned into the pCR4-TOPO vector (Cat N°. K4575-40, Invitrogen) and sequenced. The consensus sequence resulting from the sequencing of both PCR products is herein represented by SEQ ID NO: 1111 and is referred to as the partial sequence of the NL023 gene. The corresponding partial amino acid sequence is herein represented as SEQ ID NO: 1112.

C. dsRNA Production of *Nilaparvata lugens* Genes

[0391] dsRNA was synthesized in milligram amounts using the commercially available kit T7 Ribomax™ Express RNAi System (Cat. Nr. P1700, Promega). First two separate

single 5' T7 RNA polymerase promoter templates were generated in two separate PCR reactions, each reaction containing the target sequence in a different orientation relative to the T7 promoter.

[0392] For each of the target genes, the sense T7 template was generated using specific T7 forward and specific reverse primers. The sequences of the respective primers for amplifying the sense template for each of the target genes are given in Table 4. The conditions in the PCR reactions were as follows: for NL001: 4 minutes at 94° C., followed by 35 cycles of 30 seconds at 94° C., 30 seconds at 60° C. and 1 minute at 72° C., followed by 10 minutes at 72° C.; for NL002: 4 minutes at 94° C., followed by 35 cycles of 30 seconds at 94° C., 30 seconds at 60° C. and 1 minute at 72° C., followed by 10 minutes at 72° C.; for NL003: 4 minutes at 94° C., followed by 35 cycles of 30 seconds at 94° C., 30 seconds at 66° C. and 1 minute at 72° C., followed by 10 minutes at 72° C.; for NL004: 4 minutes at 95° C., followed by 35 cycles of 30 seconds at 95° C., 30 seconds at 54° C. and 1 minute at 72° C., followed by 10 minutes at 72° C.; for NL005: 4 minutes at 95° C., followed by 35 cycles of 30 seconds at 95° C., 30 seconds at 57° C. and 1 minute at 72° C., followed by 10 minutes at 72° C.; for NL006: 4 minutes at 95° C., followed by 35 cycles of 30 seconds at 95° C., 30 seconds at 54° C. and 1 minute at 72° C., followed by 10 minutes at 72° C.; for NL007: 4 minutes at 95° C., followed by 35 cycles of 30 seconds at 95° C., 30 seconds at 51° C. and 1 minute at 72° C., followed by 10 minutes at 72° C.; for NL008: 4 minutes at 95° C., followed by 35 cycles of 30 seconds at 95° C., 30 seconds at 54° C. and 1 minute at 72° C., followed by 10 minutes at 72° C.; for NL009: 4 minutes at 95° C., followed by 35 cycles of 30 seconds at 95° C., 30 seconds at 54° C. and 1 minute at 72° C., followed by 10 minutes at 72° C.; for NL010: 4 minutes at 95° C., followed by 35 cycles of 30 seconds at 95° C., 30 seconds at 54° C. and 1 minute at 72° C., followed by 10 minutes at 72° C.; for NL011: 4 minutes at 95° C., followed by 35 cycles of 30 seconds at 95° C., 30 seconds at 53° C. and 1 minute at 72° C., followed by 10 minutes at 72° C.; for NL012: 4 minutes at 95° C., followed by 35 cycles of 30 seconds at 95° C., 30 seconds at 53° C. and 1 minute at 72° C., followed by 10 minutes at 72° C.; for NL013: 4 minutes at 95° C., followed by 35 cycles of 30 seconds at 95° C., 30 seconds at 55° C. and 1 minute at 72° C., followed by 10 minutes at 72° C.; for NL014: 4 minutes at 95° C., followed by 35 cycles of 30 seconds at 95° C., 30 seconds at 51° C. and 1 minute at 72° C., followed by 10 minutes at 72° C.; for NL015: 4 minutes at 95° C., followed by 35 cycles of 30 seconds at 95° C., 30 seconds at 55° C. and 1 minute at 72° C., followed by 10 minutes at 72° C.; for NL016: 4 minutes at 95° C., followed by 35 cycles of 30 seconds at 95° C., 30 seconds at 57° C. and 1 minute at 72° C., followed by 10 minutes at 72° C.; for NL018: 4 minutes at 95° C., followed by 35 cycles of 30 seconds at 95° C., 30 seconds at 55° C. and 1 minute at 72° C., followed by 10 minutes at 72° C.; for NL019: 4 minutes at 95° C., followed by 35 cycles of 30 seconds at 95° C., 30 seconds at 54° C. and 1 minute at 72° C., followed by 10 minutes at 72° C.; for NL021: 4 minutes at 95° C., followed by 35 cycles of 30 seconds at 95° C., 30 seconds at 55° C. and 1 minute at 72° C., followed by 10 minutes at 72° C.; for NL022: 4 minutes at 95° C., followed by 35 cycles of 30 seconds at 95° C., 30 seconds at 53° C. and 1 minute at 72° C., followed by 10 minutes at 72° C.; for NL023: 4 minutes at 95° C., followed by 35 cycles of 30 seconds at 95° C., 30 seconds at 52° C. and 1 minute at 72° C., followed by 10 minutes at 72° C.